Instructions

QUINNIPIAC RIVER FUND GRANT AWARD - FINAL REPORT QUESTIONS

This form is to be completed by all nonprofit organizations that received a grant through the Quinnipiac River Fund.

Grant Details

Grant Details

Organization Name

University of New Haven

Grant Description

to support the study of the prevalence of the ulvoid bloom in the New Haven Harbor.

Total Grant Amount

9,675.00

Report Questions

1. List the specific objectives/outcomes of the project and tell how they were met during the grant period. Also, provide an update on any special conditions of the grant (if applicable).

In this project, we aimed to understand why the green alga Ulva compressa is such a prevalent bloom former. We collected live material from several sites in New Haven Harbor and the brackish portion of the Quinnipiac River: Clifton Street Boat Launch, Lighthouse Point, Morris Cover, Long Wharf, Sandy Point, and Bradley Point. We used this material to address the following objectives:

1) Determine the physiological response (through measurements of photosynthetic efficiency using Pulse Amplitude Modulation (PAM) fluorometry) of Ulva compressa to the environmental stressors temperature, salinity, and copper.

We used a series of mesocosm experiments to expose U. compressa to a single stressor at a variety of levels at one time. Depending on the stressor, experiments were run between six and fourteen days; shorter experiments were stopped early due to death of tissues in most treatments. The physiological response was assessed through measurements of photosynthetic efficiency (measured as the maximum electron transport rate, ETRmax) using a JUNIOR-PAM (Walz Photosynthesis Instruments, Germany). These measurements allow for non-destructive sampling of the algae over the course of the experiments (Figure 1). Ulva samples exposed to the temperatures 20□C, 25□C, and 30□C gradually declined over the experiment, although were still viable after two weeks in culture (Figure 1A). Samples at 20□C and 25□C responded in a similar fashion, while those at 30□C decreased the most in their photosynthetic activity.

Ulva exposed to varying levels of salinity, from 5ppt – 30ppt, were the most stable of the three stressors tested. ETRmax fluctuated and showed a small decline by the end of the experiment, with the 30ppt treatment declining the most (Figure 1B). Ulva is known for its broad salinity tolerance, so this result was expected but the lower performance of the 30ppt treatment was somewhat surprising as these samples were collected from New Haven Harbor, which generally has a salinity of ~28ppt.

The most dramatic response in our experiments was to the copper stressor. Only the 0ppm and 2ppm treatments survived until the end of the experiment, with samples in the highest concentration 12ppm dying after one day (Figure 1C). Even the 2ppm treatment was in marked decline by the last day.

These results indicate that Ulva compressa is able to acclimate to a wide range of salinities, which likely contributes to its broad distribution in marine and brackish waters. It did not respond well to changes in temperature, although it was able to survive in increased temperature for two weeks. Any level of copper was toxic to the algae, with material showing a gradient in its decline over the increased concentrations. Since Ulva is efficient in its uptake of chemicals in the surrounding environment, this indicates that the algae are particularly susceptible to heavy metals and legacy pollutants like copper.

2) Test the hypothesis that there is an epigenetic response to environmental stressors that is regulated through DNA methylation, as measured using the methylation-sensitive amplified polymorphism (MSAP) procedure.

Following the Methylation-sensitive amplification polymorphism (MSAP) protocol as outlined in Baurens et al. (2008), we were able to obtain profiles for samples at the beginning and end of each experiment in order to determine the percentage of loci that were methylated. All samples collected from the field had some degree of methylation, ranging from 30% - 67%. Change in methylation, which would indicate a change in gene expression, varied among the stressor being tested. Ulva exposed to different temperatures showed almost no change in methylation percentage from day 0 compared to the last day of the experiment, for all treatments. These samples also showed a decline in their photosynthetic health, indicating that they were not able to regulate their response to this stressor, at least in this experiment.

In the salinity experiment, the lower salinities (5,10, and 15ppt) had a decrease of 15% methylation, while higher salinities (25 and 30ppm) had the great changes in methylation patterns of 40% and 48%, respectively. These samples had only a small decline in photosynthetic health over the course of the experiment but were able to maintain physiological activity. This together with the changes in methylation support our hypothesis.

In the copper experiment, the 0ppm control had no change, while the 2ppm treatment showing the greatest change in methylation with a 45% increase on the last day of the experiment compared to day 0. The 6ppm treatment had a 28% increased on the last day compared to day 0. For the remaining treatments, material was so degraded that DNA extraction and subsequent MSAP analysis was not possible. The 2ppm treatment was the only copper concentration where Ulva was able to maintain some physiological health, and this greatest change in methylation pattern supports our hypothesis.

Our data, especially from the salinity and copper experiments, support our hypothesis that there is an epigenetic response to environmental stressors that is regulated through DNA methylation. The results from the temperature experiment are inconclusive and need to be repeated. In other temperature experiments conducted in the lab conducted on Ulva samples we have seen better acclimation to temperature change, so we need to examine this stressor further to ascertain the physiological response in Ulva from New Haven Harbor.

3) Assess the morphological response of Ulva compressa to the environmental stressors temperature, salinity, and copper: is tube or blade morphology predetermined, or will it change in response to a new environment?

We did not see any changes in morphology in response to temperature, salinity, or copper. Additionally, we were only able to collect the blade form of this species during this study, despite collecting the tube form on occasion in previous years. It is possible that our experiments did not run long enough to see any morphological change. It is also possible that one would need to use material cultured from zoospores to show changes.

2. Please share your successes, challenges and any lessons learned through the implementation of your project. Were there any unintended consequences or lessons learned that may affect how you operate your program moving forward?

The greatest challenge with this project was the COVID pandemic hitting right at the beginning. Our campus was in lockdown and all research activities were halted for the spring semester, which delayed our field collections and lab experiments. Even so, we were able to obtain approval and got started with work in the summer. Field and lab research while social distancing was a challenge, but we overcame it by following our university’s safety protocols at the time.

Another challenge was implementing the MSAP protocol. This is a multi-step lab protocol with several potential pitfalls that required trouble-shooting. We were able to gather advice from colleagues who are experts in the field of molecular biology and subsequently made modifications to our lab protocols that resulted in better and more consistent results across samples. This improved protocol will prove useful for future projects where we wish to assess methylation in samples, turning this challenge into one of the success stories from this project.

3. What are the opportunities and needs of your organization as it continues to move forward with its work to positively impact the Quinnipiac River?

This project helped in our understanding of why Ulva compressa is such a prevalent bloom former: it is able to acclimate rapidly to environmental stressors, likely due to an epigenetic response. Further work is needed to confirm these findings through replication of experiments, expansion of treatments, and testing on other species of bloom-forming Ulva. With the establishment of an MSAP protocol that reliably works for our study system, we will be able to address related questions in the future. Additionally, we have established sampling locations around New Haven Harbor, where Ulva blooms reoccur every summer. By studying these sites over time we can better understand bloom dynamics within the harbor itself, while drawing broader conclusions about these bloom forming algae that can be applied to other locations.

Attachments

Financial information (required): Please provide a detailed accounting of how the specific grant dollars were spent based on the budget submitted in the grant application.

Detailed Accounting

PV123_Internal Financial Report_02.09.24.xlsx

Pictures (optional): Please attach 1 to 3 pictures of activities that have occurred throughout the grant period (with a description for each) as a result of grant funding. All pictures should be submitted in JPEG format and may be uploaded to www.thequinnipiacriver.com and used in Foundation publications.

Picture 1

MSAP_Ulva_fig1.jpg

Description

Figure 1. The physiological response of Ulva compressa measured as the maximum photosynthetic electronic transport rate (ETRmax) over the course of mesocosm experiments. Samples were exposed to varying (A) temperature, (B) salinity, and (C) copper.

Picture 2

Description

Picture 3

Description